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DEVELOPMENT OF IN VITRO ISOLATED PERFUSED PORCINE SKIN FLAPS FOR STUDY OF PERCUTANEOUS ABSORPTION OF XENOBIOTICS

ANNUAL REPORT

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19. ABSTRACT

Interspecies comparisons of cutaneous anatomy and physiology suggest that the weanling pig is a suitable surrogate for human skin; however, very few investigations of percutaneous absorption phenomena have been conducted in pigs. This study examined the radiolabel excretion patterns after intravenous (IV) and topical administration of six ^{14}C-labeled compounds in weanling Yorkshire sows. These data were required as a baseline to compare xenobiotic absorption in the isolated perfused porcine skin flap (IPPSF) fully described in the first Annual Report (Riviere, J. E., Bowman, K. F., and Monteiro-Riviere, N. A., Development of In Vitro Isolated Perfused Porcine Skin Flaps for Study of Percutaneous Absorption of Xenobiotics, USAMRDC, DAMD17-84-C-4103, November 1, 1986). IV doses (200 µg) of malathion (M), parathion (P), caffeine (C), and benzoic acid (B) were primarily excreted into the urine (>80%), while greater fractions of testosterone (T, 72%) and progesterone (R, 35%) were excreted into the feces. Percutaneous absorption from topical doses in ethanol of 200 g (40 µg cm^{-2}) occurred in the following rank order: B (25.7%) > R (16.2%) > C (11.8%) > T (8.8%) > P (6.7%) > M (5.2%). Because fecal clearances were greater after topical administration for four of the six compounds (B, C, P, and T, $p<0.05$), dermal absorption calculated from urinary excretion alone underestimated the true values by 8-30%. Although the reason for this increased fractional fecal excretion is not certain, first-pass cutaneous biotransformation which is known to occur for P and T may have contributed to the altered excretion profile of compounds applied topically.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

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INTRODUCTION

One of the most promising areas for pharmacological and toxicological investigation involves the transport of chemical substances through skin, or percutaneous absorption. It is universally accepted that skin is a major portal of entry into the body for drugs and environmental agents, thus skin must be considered a target organ for both drugs and toxins. Therapeutic strategies are available in which drugs applied topically may penetrate in sufficient amounts to treat problems localized to the skin and, more recently, transdermal drug delivery systems have been developed to provide a convenient, noninvasive method for infusing drugs systemically.

In order to fully characterize the interaction of skin with topical agents, a better understanding of the mechanisms of percutaneous absorption is necessary, particularly with regard to the animals used as surrogates for human percutaneous absorption studies. Comparative anatomy of animal and human skin suggests that certain pink-skinned porcine species are more dermatologically similar to humans than most other animals studied. Similarities between human and pig skin in pelage density and thickness of the various dermal layers (1-3), cutaneous vasculature and microcirculation (4-6) biochemistry and cellular metabolism (7-9), histochemistry and enzyme distribution (10,11), epidermal and surface lipid content (1,10,12-15), and ultrastructure (16,17) lend support to this hypothesis.

Interspecies comparisons of percutaneous absorption rates and penetration characteristics for a wide range of compounds both *in vivo* (18-23) and *in vitro* (24-26) all suggest that weanling or miniature pig and human skin are equivalent when studied under identical conditions. Although the rhesus monkey may represent an even better model for human skin than weanling pigs (27-31), these animals are much more expensive and difficult to handle, in addition to the possibility that animal rights legislation may eventually outlaw their use in research projects altogether.

Recently, there has been some concern that the underlying assumptions inherent in the protocol used to assess percutaneous absorption *in vivo*, which was standardized over 20 years ago (32), may not always be valid. In particular, the exclusive use of urinary excretion of radiolabel following intravenous (IV) and topical administration may not provide accurate estimates of percutaneous absorption for some compounds. In addition to the obvious need to identify the penetrating species, given the known capacity of skin for biotransformation reactions (33-35), there is a growing body of evidence that topical and IV excretion profiles or, more specifically, the urine:fecal excretion ratios may differ (36-40). Indeed, these two phenomena may be related, since an analogous and well known "first-pass effect" occurs after oral administration and the route of xenobiotic excretion

often differs from that which is observed following a parenteral dose.

The present investigation of percutaneous absorption in weanling Yorkshire pigs was undertaken for several reasons. First, a data base for topical absorption of several chemical classes is needed to validate use of the isolated perfused porcine skin flap (IPPSF) for examining cutaneous pharmacology and toxicology in vitro (7,41,42). The development of the IPPSF was fully described in the first Annual Report (43) for this contract. The validation of compound absorption in the IPPSF with the in vivo data reported herein will be presented in the Final Report. Compounds chosen for this study represent a wide range of lipid-water partition coefficients (44) and have been examined previously in pigs at other sites and other applied surface concentrations (19,20,22,23,25). Finally, although it is recognized that pigs may provide the most reliable surrogate for human skin, surprisingly few studies have been conducted using pigs. Factors known to affect percutaneous absorption in other animals and man, such as applied surface concentration, anatomic location of the application site, vehicle and solvent effects, and cutaneous biotransformation have not been examined in pigs, which makes it difficult to extrapolate from one study to another. Urine and fecal clearances after both IV and topical routes of administration will be compared to determine whether it is necessary to collect total excrement for accurate measurements of percutaneous absorption in vivo.

MATERIALS AND METHODS

[1-methyl-^{14}C]Caffeine (specific activity = 47.5 mCi/mmol) and [7-^{14}C]benzoic acid (specific activity = 19.3 mCi/mmol) were purchased from New England Nuclear (Boston, MA). [2,3-^{14}C]Malathion (specific activity = 37 mCi/mmol), [ring 2,6-^{14}C]Parathion (specific activity = 21 mCi/mmol), [ring 4-^{14}C]Progesterone (specific activity = 56 mCi/mmol), and [ring 4-^{14}C]Testosterone (specific activity = 56.9 mCi/mmol) were purchased from Amersham (Arlington Heights, IL). Unlabeled (cold) caffeine (C), benzoic acid (B), testosterone (T), and progesterone (R) were purchased from Sigma (St. Louis, MO). Cold malathion (M) and parathion (P) were purchased from Chem Service, Inc. (West Chester, PA). The radiochemical purity of all ^{14}C compounds was certified by manufacturer's thinlayer chromatographic analyses to be >97%. Female weanling Yorkshire swine, weighing approximately 20 kg each (17.8 $\pm$ 0.4, N = 64), were obtained for use in all studies. Pigs were acclimated for at least 1 week before entering into the study, housed at standard temperature (72°F) and light-dark cycle (12:12 hr), and were fed 15% pig and sow pellets, 2 lb/day (Wayne Feed Division, Chicago, IL) and approximately 2 L water/day.

Dosing solutions were prepared for IV injection by addition of cold compound in ethanol solution to the radiolabeled

material. This was further diluted in sterile physiological (pH = 7.4) saline to a final concentration of 0.02 mg/ml and a radioactive concentration of 1 μCi/ml. All IV solutions were prepared within 1 week of use. Pigs were weighed and anesthetized by intramuscular injection of 4 mg/kg xylazine (Rompun; Miles/Bayvet, Shawnee, KS) and 16 mg/kg ketamine (Ketaset; Bristol Laboratories, Syracuse, NY). Ten milliliters of the IV dosing solution was administered to each pig by bolus injection into an ear vein. Pigs were immediately placed into metabolism cages, constructed so that total urine and feces could be collected separately. Urine was collected at 6, 12 (where available), and 24 hr and daily thereafter for a total of 6 days. The volume of each collection was measured and a small sample was stored at -20°C until analysis. Feces were collected daily for 6 days and the total sample, placed in plastic bags, was also stored at -20°C. At the end of the study, pigs were euthanized with 60-80 mg/kg (IV) pentobarbital solution (Uthol, Butler, Columbus, OH). Liver, both kidneys, and spleen were removed and weighed. Small samples of each, along with samples of lung, colon, skeletal muscle, and skin, were stored as described above. For two pigs, one receiving benzoic acid and another dosed with malathion, a cannula was inserted through a 12 gauge needle into the jugular vein and blood samples (1-ml each) were taken at 15, 30, 45, 60, and 90 min and at 2, 3, 4, 6, 8, 12, 24, and 48 hr for pharmacokinetic disposition studies.

For topical administration, dosing solutions were prepared by diluting cold and radiolabeled compound in 100% ethanol to a final concentration of 1 mg/ml and 50 μCi/ml. Pigs were weighed and anesthetized as described for the IV studies and a topical application procedure similar to that described previously (23) was followed. The abdominal area on each pig was lightly shaved with electric clippers, taking care not to damage the skin surface (29,30). The intended area to be dosed (5 cm x 1 cm) was marked and a small foam rubber patch (10 cm x 5 cm x 2 cm) with the center cut out was glued to the skin immediately adjacent to this area. Neither the glue nor the patch touched the area to be dosed. Using a micropipetter (Hamilton, Reno, NV), 200 μl of the ethanolic dosing solution was applied to the site to provide an applied surface concentration of 40 μg cm^{-2}. A nylon screen and non-occlusive gauze pad were used to cover the foam patch and were held in place by wrapping the pig's midsection with elastic tape (Elasticon; Johnson & Johnson, New Brunswick, NJ). Pigs were then placed in the metabolism cage. Excrement collections and tissue samples at termination of the study were the same as for the IV pigs, except that the foam patch, dosed site, and adjacent skin were also collected and stored separately.

Because the coefficient of variation in the radiolabel concentration determined from a single fecal collection chosen at random was 53% (N = 5 replicates) without prior preparation, it was deemed necessary to homogenize all feces to provide a well mixed sample for accurate radiolabel determination. Each total daily fecal collection from both IV and topically dosed pigs was

individually weighed and ground into a paste in a Waring commercial blender, with addition of 100-300 ml physiological saline (pH = 7.4) to facilitate grinding. A small amount of the homogenate was collected in a plastic vial and stored until assay. Coefficients of variation checked in two randomly selected samples were significantly lower, averaging 8-11% (N = 5).

Aliquots of urine (500-800 µl), plasma (500 µl), red blood cells (200 µl), tissue samples from the internal organs (0.3-0.8 g), and fecal homogenate samples (0.6-0.8 g) were oxidized without further preparation in an open-flame tissue oxidizer (Model 306; Packard Instrument Co., Downers Grove, IL). The trapped radiolabel was measured using a liquid scintillation counter (LSC) equipped with automatic external standard quench and color correction (1219 Rackbeta; LBK Wallac, Turku, Finland). Each skin sample was weighed and immersed in a flask containing tissue solubilizer (BTS-450; Beckman Instruments, Fullerton, CA), in amounts of 5-10 ml/g wet tissue weight. The flask was placed in a water bath overnight and maintained at 42°C with shaking (80 oscillations/min). Aliquots of the solubilized skin (100 µl) were added to 10 ml scintillation cocktail (Scintiverse Bio-HP; Fisher Scientific, Fair Lawn, NJ) and counted by LSC as described above. Several samples of the application sites and patch skin were also directly oxidized and counted to assure that solubilization did not affect total recoveries. No significant differences were observed between the two procedures. Foam rubber patches were extracted in an 80:20 (v/v) ethanol/methanol mixture. Samples of the extract (500 µl) were added to 10 ml of scintillation cocktail and counted as before.

Whole-body residues and excretion recoveries were calculated by multiplying radiolabel concentrations by measured urine volumes and fecal and organ weights (liver, kidneys, spleen), or by the estimated organ weights for lung, colon, skin, and muscle (45). Skin samples and patch extract recoveries were calculated separately. A two-compartment pharmacokinetic model was fitted to the blood and plasma concentrations and clearance (Cl), volume of distribution (Vc and Vss), and half-life ($T_{1/2}$) were calculated according to standard formulas (45). Percutaneous absorption, expressed as a percent of total dose, was corrected for incomplete excretion using the following formula (32):

$$\text{Corrected absorption} = \frac{\text{Topical excretion}}{\text{IV excretion}} \times 100\%$$

Percutaneous absorption estimates based on urine alone or urine + feces for each route were determined to compare the two methods for calculating fractional absorption (40). Elimination rates (K) for IV and topical excretion were estimated from simple linear regression of a sigma-minus plot of log [amount remaining to be excreted] vs. time (46). All data in the tables and figures are

reported as means ± SE and inferences were based on Student's t-statistic.

RESULTS

Although hematocrits (PCV), measured in 15 randomly selected pigs, increased an average of 16% (initial PCV = 30.9 ± 0.5, terminal PCV = 36.0 ± 1.0), indicating a slight dehydration due to restricted water intake, this was not considered clinically significant and was independent of the route of administration. Other than a slight and transient erythema which occurred in the skin of all pigs given T topically, no toxicological or pharmacological reactions to the compounds used were noted and internal organs examined at necropsy all appeared normal.

The overall disposition of the radiolabel for the two routes of administration is shown in tables 1 and 2. Total recoveries following the IV doses were high for B and C (near 90%), somewhat lower for the steroids (60-80%), and lowest for the more volatile organophosphates (50-60%). Since fat residues were not measured, the lower recoveries of steroids may be due to storage in body fat. In addition, it is impractical to collect expired air in pigs, which may account for the failure to retrieve a larger fraction of the injected organophosphates. As can be seen in table 1, carcass totals, obtained by summing the individual organs assayed, were negligible for all but the organophosphates. Individual organ levels were near the lower limits of detection in most cases, although small amounts of C were found in the liver (0.3%), P in the skin (0.7%), and R in the lungs (0.2%). Significant residues of M were found in all organs assayed, particularly the skeletal muscle which contained over 11% of the injected M. For the nonsteroidal compounds, urinary excretion was much greater than fecal excretion, averaging over 80% of the total amounts excreted during the 6 day collection period. On the other hand, 25-70% of the total steroid excretion occurred by the fecal route.

For the topical route of administration, a different pattern emerged. As expected, most (50-95%) of the recovered radiolabel was found in the patch surrounding the application site and in the skin sample comprising the site itself. A small percentage of the dose was occasionally found in the skin surrounding this site. As demonstrated in the low carcass totals, individual organs retained levels which were barely detectable for any of the compounds. Although urinary excretion again predominated for all except the steroids, fecal clearance represented a greater fraction of total elimination in most pigs by the topical route of administration.

This latter finding is illustrated in figure 1, in which the fecal clearances, expressed as a percentage of total for each route, are compared. As can be seen, for four of the six compounds studied (B, C, P, and T), the fraction of total radiolabel excreted by the fecal route was greater after topical

Table 1. Radiolabel recovery (% dose) following IV administration of ^{14}C-labeled compounds to pigs[a,c]

Compound	Urine	Feces	Carcass	Total Excretion[b]	Total Recovery
B	84.5 ± 9.0	4.6 ± 1.2	0.1 ± 0.05	89.1 ± 9.3	89.3 ± 9.3
C	68.2 ± 2.9	16.6 ± 4.1	1.5 ± 0.5	84.8 ± 3.2	86.3 ± 3.2
M[d]	32.2 ± 3.3	4.9 ± 0.7	13.1 ± 2.7	37.1 ± 2.6	50.2 ± 3.5
P	57.8 ± 2.3	2.0 ± 0.4	1.2 ± 0.6	59.9 ± 2.1	61.1 ± 2.0
R	22.1 ± 4.0	40.0 ± 4.2	0.6 ± 0.1	62.1 ± 4.4	62.7 ± 4.4
T	54.4 ± 3.6	21.2 ± 2.7	0.7 ± 0.3	75.7 ± 2.2	76.3 ± 2.4

[a]Total dose = 200 µg, 10 µCi/pig (N = 4).

[b]Correction factor for topical excretion studies.

[c]Mean ± SE.

[d]N = 3.

Table 2. Radiolabel recovery (% dose) following topical administration of ^{14}C-labeled compounds to pigs[a,d]

Compound	Urine	Feces	Carcass	Patch	Dosed Skin	Patch Skin[b]	Total Recovery
B[c]	20.0 ± 2.3	2.9 ±0.3	0.8 ±0.4	40.2 ± 0.3	12.2 ± 1.0	9.1 ± 2.1	85.4 ± 4.2
C	5.8 ± 0.4	4.2 ±1.0	2.7 ±1.0	75.3 ± 4.9	2.8 ± 0.8	1.9 ± 0.2	92.7 ± 3.3
M[c]	1.7 ± 0.3	0.2 ±0.1	0 0	86.2 ± 7.6	2.1 ± 0.1	2.0 ± 1.2	92.2 ± 8.6
P	3.1 ± 0.6	0.9 ±0.2	1.3 ±1.0	77.2 ± 1.8	0.6 ± 0.1	0.6 ± 0.3	83.6 ± 1.2
R[c]	2.6 ± 0.6	7.4 ±1.3	0.3 ±0.1	58.2 ± 0.8	8.6 ± 1.0	7.2 ± 0.2	94.5 ± 11.9
T	3.8 ± 1.7	2.8 ±0.8	0.7 ±0.2	81.2 ± 9.0	17.6 ± 4.9	4.7 ± 1.7	111.0 ± 4.5

[a]Total dose = 200 µg, 10 µCi/pig (N = 4).

[b]Skin taken from area immediately adjacent to dosed site out to patch boundaries.

[c]N = 3.

[d]Mean ± SE.

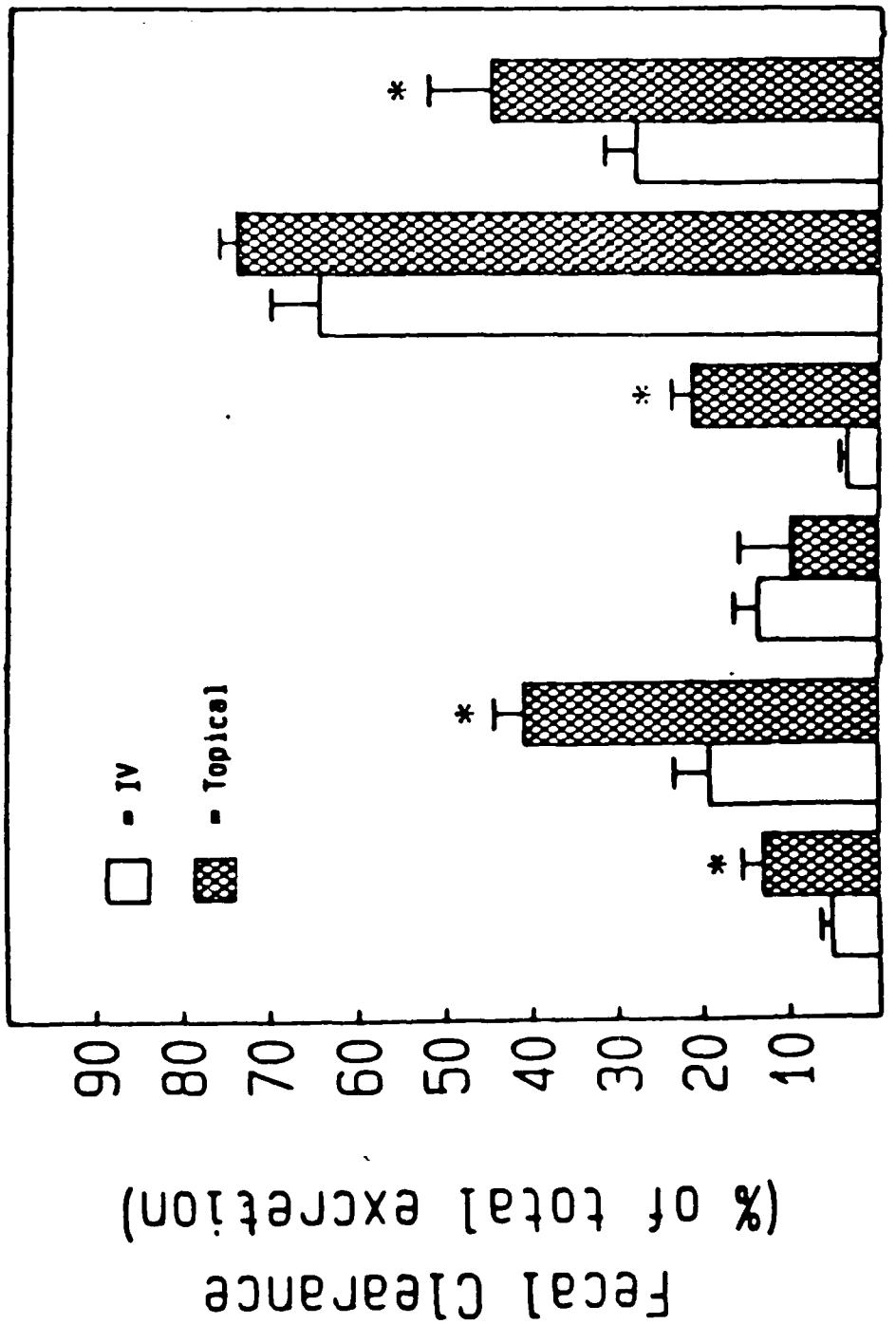


Figure 1. Comparison of fractional fecal excretion after IV (unfilled bars) and topical (hatched bars) administration. Error bars represent 1 SE and the asterisks denote significant differences between routes ($p < 0.05$).

administration than by IV dosage ($p<0.05$). Only M, for which the fecal clearance declined slightly following topical application, had almost identical elimination patterns for both routes. The rank order of corrected skin absorption was as follows: B (25.7%) > R (16.2%) > C (11.8%) > T (8.8%) > P (6.7%) > M (5.2%). With the exception of the reversal of C and R, the rank order can also be approximately expressed in terms of the classes as: well absorbed organic acid/base compounds > steroid hormones > organophosphate insecticides.

The calculated excretion rates are presented in table 3. As expected, these rates were generally greater following IV administration, with statistically significant differences ($P<0.05$) seen for four of the six compounds. Elimination half-lives ($T_{1/2}$), calculated using the mean elimination rates in table 3, ranged from 15 to 20 hr following IV administration. The bulk of the radiolabel appeared in the urine and feces during the first 24 hr after dosing, with the highest rates during the first collection interval (0-6 hr, urine alone) for all compounds. IV elimination was generally biphasic, as exemplified by B, with a rapid elimination during the first day ($T_{1/2}$ = 2.5 hr) and a much slower rate over the next 5 days ($T_{1/2}$ = 19.6 hr). Pharmacokinetic parameters calculated from the blood data from a single pig are in agreement, with Cl = 7.35 ml/min/kg, Vss = 0.373 L/kg, Vc = 0.329 L/kg, and $T_{1/2}$ = 2.43 hr. The corresponding values for the serum available from one pig given M by same route are: Cl = 0.90 ml/min/kg, Vss = 2.60 L/kg, Vc = 0.309 L/kg, and $T_{1/2}$ = 41.35 hr. Whereas B distributes almost entirely within the central compartment, M has a much greater apparent volume of distribution, which may be the reason for the difference in tissue residues seen in table 1. Biphasic elimination patterns were not seen in the sigma-minus plots of topical excretion data, indicating that the elimination rates are masked kinetically by the much slower absorption rate processes. The period of peak absorption occurred during the second collection interval (6-24 hr) for all compounds except R (48-72 hr), and $T_{1/2}$ values ranged from 18 to 35 hr.

Finally, because the finding of an altered excretion profile has a potential impact on the interpretation of percutaneous absorption estimates derived from excretion methodology, a comparison of estimates using urine alone with those obtained from combined excrement is shown in figure 2. Although the difference in percutaneous absorption totals given by the two methods is only statistically significant for C ($p<0.05$), the small number of pigs used for each route and compound and the relatively high variability in the data obscure the overall significance of this finding. In the present study, percutaneous absorption totals obtained using urinary excretion alone underestimated the "true" value, assuming clearance by routes other than urine and feces is negligible, by approximately 8-30%.

Table 3. Elimination rates (K) obtained from total radiolabel excretion after IV and topical administration[a,b]

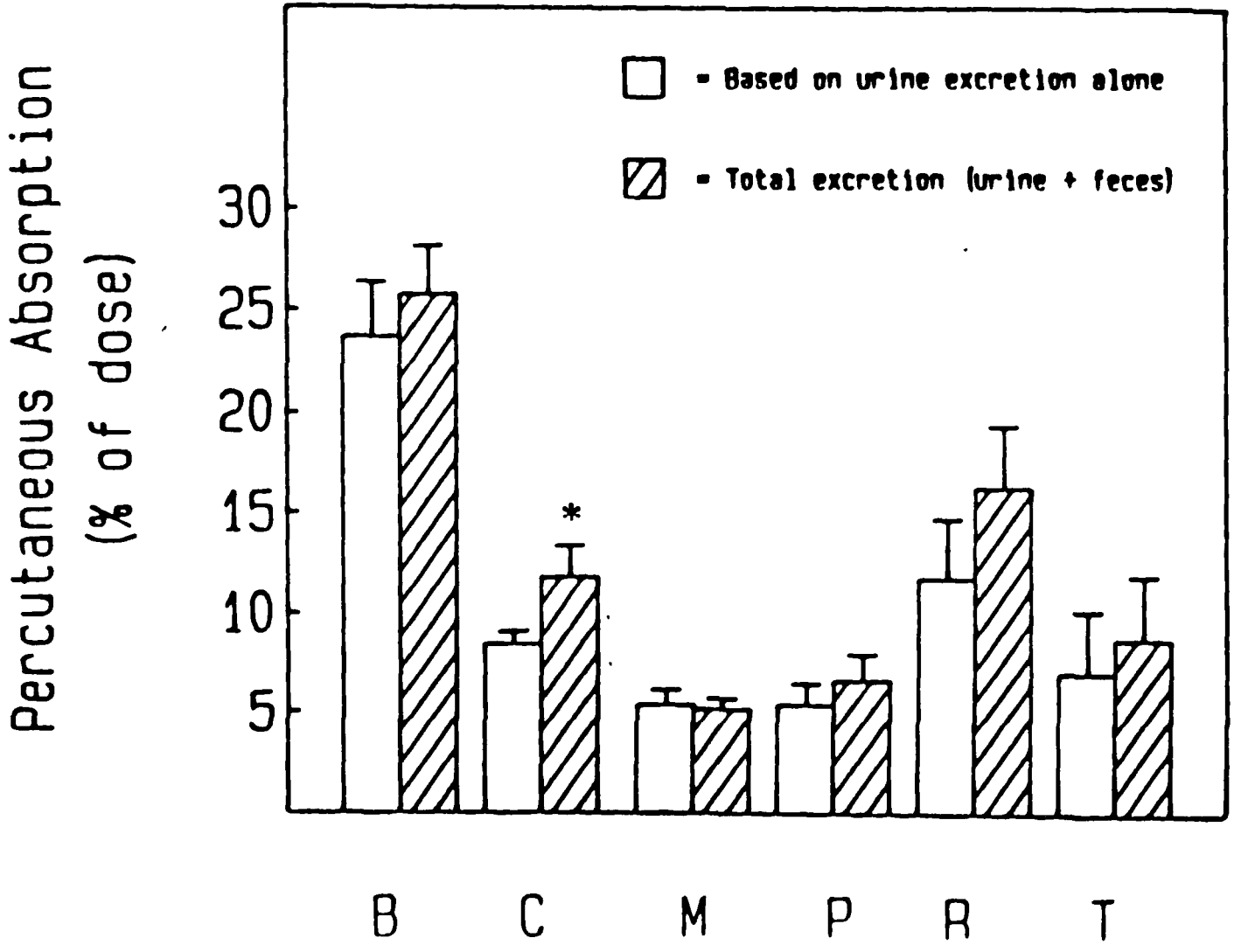
Compound	IV K	Topical K
B	0.0354 ± 0.0048 (4)	0.0289 ± 0.0024 (3)
C	0.0467 ± 0.0035 (4)	0.0240[c] ± 0.0015 (4)
M	0.0377 ± 0.0017 (3)	0.0381 ± 0.0082 (3)
P	0.0386 ± 0.0032 (4)	0.0276[c] ± 0.0029 (4)
R	0.0368 ± 0.0013 (4)	0.0200[c] ± 0.0003 (3)
T	0.0414 ± 0.0061 (4)	0.0223[c] ± 0.0024 (4)

[a]Total dose for each route = 200 µg, 10 µCi/pig.

[b]Mean ± SE (N).

[c]Significantly lower than IV K ($p<0.05$).

Figure 2. Corrected percutaneous absorption estimates calculated by two different methods: Using urinary excretion alone (unfilled bars) or using both urine and fecal excretion (hatched bars). Error bars represent 1 SE and an asterisk denotes significant difference between the two methods. ($p < 0.05$).



DISCUSSION

Percutaneous absorption totals in the present study were generally lower than values reported by others in pigs, despite the similarities in overall rank order, both by compound and chemical class (18-20,22,23). The high degree of inter-laboratory variability in excretion of these compounds, given either parenterally or topically to pigs, renders absolute comparisons problematic. However, there are several factors known to affect percutaneous absorption in vivo, which may help explain these conflicting results. Variables such as applied surface concentration, the vehicle used, or the anatomical site onto which topicals are applied have not been evaluated in pigs, although their relative effects on percutaneous absorption are known in other species and should be qualitatively similar in pigs.

A major factor affecting percutaneous absorption in vivo is the applied surface concentration, for which an inverse relationship with fractional (%) absorption has been demonstrated in both humans and rhesus monkeys (31,47-50). A similar relationship for finite doses applied in vitro has also been documented (50). It is reasonable to assume that the higher topical doses applied in this study contributed to the lower percutaneous absorption totals, expressed as a percent of total dose, reported here, particularly since the overall rank orders were in agreement.

The application site may also be involved, since an area on the dorsum has traditionally been the site of choice for animal studies. Regional variations in percutaneous absorption are reasonably well known in humans (51-55). However, these studies are rare in other mammalian species except for the rhesus monkey, which appears to have similar rank orders as man for certain compounds (56-58). There is also some evidence that the abdominal region in rats is more permeable than the back (52,59). Although the ratio of pig-to-human absorption for some compounds is similar to back-to-forearm ratios in man (21,22,55) other application sites have not been examined in pigs. Further studies of various anatomic regions and applied doses are clearly warranted if these animal data are to have any relevance in human dermal risk assessment. The ventral abdomen was chosen in the present study because it is the location from which porcine skin flaps, useful for examining cutaneous pharmacology and toxicology in vitro, are surgically removed (7,41,42).

The dependence of the urine-fecal excretion ratios on route of administration shown in this study for B, C, P, and T has not been demonstrated previously. Examples of altered excretion profiles are available for both T (40) and polychlorinated biphenyls (39) in guinea pigs, 1,3-diphenylguanidine in rats (37), and dimethylbenzanthracene in mice (36). In this study, the injected dose was systemically bioavailable in amounts 4-20 times

greater than the amounts absorbed after topical application, which, when coupled with slower presentation to eliminating organs by the latter route, might explain the route-dependent excretion pattern observed. Moreover, incomplete recovery totals of radiolabel from IV doses of organophosphates suggest that volatile metabolites and expired $^{14}CO_2$ are eliminated by different mechanisms than after topical administration. However, a complete explanation for our finding of altered urine-fecal excretion ratios must lie elsewhere, since parenteral doses several orders of magnitude higher than the topical doses had little effect on radiolabel excretion in a previous study in pigs (20).

An intriguing possibility is that first-pass cutaneous biotransformation during percutaneous absorption could have occurred, followed by preferential excretion of some metabolites in the feces. Although there is no information in the literature concerning metabolism of either B or C in skin, T has been shown to be metabolized in vitro by human skin, in addition to skin from five other species-- rat, rabbit, guinea pig, mouse, and marmoset (60-62). No evidence of the bioactivation of P to its more toxic oxygenated derivative, paraoxon, was seen when rabbit, rat, cat, or human skin slices were incubated in vitro (63). However, poor P solubility in the reaction mixture, low tissue viability, or species differences in cutaneous metabolic capacity could explain why this reaction did not occur. Preliminary results in our laboratory using the IPPSF have demonstrated a substantial capacity for P metabolism during percutaneous absorption in pig skin. The majority of the ethyl acetate-extractable radiolabel which penetrated during the 8-hr studies co-migrated with paraoxon (69%) upon thin layer chromatographic separation. Unmetabolized P (24%), the product of paraoxon hydrolysis, or p-nitrophenol (5%), and a small aqueous residue (<2%) accounted for the remainder of the ^{14}C recovered in the perfusate (64,65).

The failure of others using similar in vivo methods to detect corresponding shifts in the excretion of radiolabel (20) may be a consequence of low substrate concentrations in viable cutaneous tissues, produced by the lower topical doses used. Although Michaelis-Menten enzyme kinetics has not been determined for any of the biotransformation pathways occurring in skin, substrate concentrations near the km for P in liver may result in protein binding by paraoxon, thereby preventing its release from the liver (66). Relative amounts of P (nanograms or nanomoles cm^{-2}) permeating the epidermis in this study would be 3-5 times greater than provided by the dose (4 *fg* cm^{-2}) used in previous investigations. Whether similar kinetic and protein binding phenomena occur in skin is presently unknown. It is difficult to examine cutaneous biotransformation in vivo; however, these results imply that future investigations of percutaneous absorption should focus more carefully on the metabolic fate of compounds applied topically.

In summary, percutaneous absorption of several compounds, representing three chemical classes, has been examined in vivo in pigs. Total penetration, expressed as a percentage of topical dose, was lower than in previous reports due to differences in concentration applied and the application site. The approximate rank order penetration by chemical class was: organic acids/bases > steroids > organophosphates, which was similar to that found by others in both pigs and humans. Excretion of these compounds was dependent on the route of administration, which, at the very least, violates inherent assumptions in established correction factors for using urine alone to assess percutaneous absorption. The altered excretion after topical administration may also be indicative of cutaneous biotransformation; however, further study is required to determine the metabolic capacity of skin and what effect it has on the disposition of compounds applied topically. These data will serve as the basis for assessing xenobiotic percutaneous absorption in the IPPSF.

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